Section A6.4.1 **Annex Point IIA6.4**		**Repeated dose toxicity** Subchronic oral toxicity of acrolein in rats	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Muni, I.A. (1981c) Subchronic Oral Toxicity of Acrolein (Lot No. SFSL-5893) in Rats (FIFRA Guidelines). Bioassay Systems Corporation. BSC Project No. 10258.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Baker Petrolite	
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes FIFRA 885.3600	X
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	
3.1.1	Lot/Batch number	Not available	
3.1.2	Specification	As given in Section 2	
3.1.2.1	Description	See 3.1.2.	
3.1.2.2	Purity	See 3.1.2	
3.1.2.3	Stability	See 3.1.2.	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Sprague- Dawley	
3.2.3	Source	Charles River Breeding Laboratories, Wilmington, Massachusetts	
3.2.4	Sex	Male and Female	
3.2.5	Age/weight at study initiation	Age 5-6 weeks	
3.2.6	Number of animals per group	30 animals/sex/group	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of treatment	13 weeks	
3.3.2	Frequency of exposure	5 days per week	

Section A6.4.1 **Annex Point IIA6.4**		**Repeated dose toxicity** Subchronic oral toxicity of acrolein in rats	
3.3.3	Postexposure period	None	
3.3.4	**Oral**		
3.3.4.1	Type	Gavage	
3.3.4.2	Concentration	0.0, 5.0, 0.5, 0.05 mg/kg bw	
3.3.4.3	Vehicle	Deionised water	
3.3.4.4	Concentration in vehicle	1.0, 0.1, 0.01 mg/ml	
3.3.4.5	Total volume applied	5 ml/kg	
3.3.4.6	Controls	Deionised water	
3.4	**Examinations**		
3.4.1	Observations		
3.4.1.1	Clinical signs	Yes, daily	
3.4.1.2	Mortality	Yes, daily	
3.4.2	Body weight	Yes, weekly	
3.4.3	Food consumption	Yes, weekly	
3.4.4	Water consumption	No	
3.4.5	Ophthalmoscopic examination	No	
3.4.6	Haematology	Yes 10 animals/sex/group Time point: end of study Parameters: Haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, platelet count, reticulocyte.	
3.4.7	Clinical Chemistry	Yes 10 animals/sex/group Time points: at interim and final sacrifice Parameters: Calcium, potassium, serum lactate dehydrogenase, serum glutamate pyruvate transaminase, serum glutamate oxaloacetic transaminase, glucose, blood urea nitrogen, direct and total billirubin, serum alkaline phosphate, cholesterol, albumin, globulin, total protein.	
3.4.8	Urinalysis	Yes 10 animals/sex/group Parameters: appearance, osmolality, specific gravity, pH, protein, glucose, blood, ketones, bilirubin, urobilinogen Microscopic examination made for epithelial cells, erythrocytes, leucocytes, hyaline, granular, epithelial, erythrocyte and leucocytes costs, and fat, bacteria, fungi and crystals. Sediments: Ketones, calcium oxalate, triphosphate crystals	

Section A6.4.1 **Annex Point IIA6.4**		**Repeated dose toxicity** Subchronic oral toxicity of acrolein in rats	
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	Yes Organs: Liver, kidneys, testes, ovaries, brain, heart.	
3.5.2	Gross and histopathology	Yes All dose groups Histopathology: 12 animals from control and high dose level Organs: Brain, spinal cord, pituitary, thyroid, parathyroid, thymus, oesophagus, salivary glands, stomach, small and large intestines, liver, pancreas, kidneys, adrenals, spleen, heart, trachea, lungs, aorta, gonads, uterus, female mammary gland, prostate, urinary bladder, gall bladder (mouse), lymph nodes peripheral nerve, bone marrow, skin, eyes or other	
3.5.3	Other examinations		
3.5.4	Statistics		
3.6	**Further remarks**		
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	No clinical signs were consistently seen for acrolein-treated rats.	
4.1.2	Mortality	One male at dose level, 0.5mg/kg, died due to gavage accident. No other mortalities at any dose.	
4.2	**Body weight gain**	No differences in body weight means were present between control and treated groups.	
4.3	**Food consumption and compound intake**	No differences in food consumption were present between control and treated groups.	
4.4	**Opthalmoscopic examination**		
4.5	**Blood analysis**		
4.5.1	Haematology	No significant differences were found between control and acrolein-treated animals.	
4.5.2	Clinical chemistry	No significant differences were found between control and acrolein-treated animals.	
4.5.3	Urinalysis	11 of 60 acrolein-treated animals were found to have blood in their urine.	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	No differences between mean organ weights and organ-to-body weights.	
4.6.2	Gross and histopathology	The gross lesion were mainly found in lungs, kidneys and urinary bladder and were observed by random and at low frequency. This suggests that they are incidental findings unrelated to the administration	

Section A6.4.1 **Annex Point IIA6.4**	**Repeated dose toxicity** Subchronic oral toxicity of acrolein in rats	
	of the test compounds. For histopathology, the majority of the observations described were not test material related. There were a couple of rats which showed lung lesions effected by murine respiratory viruses. The myocardial lesions were observed in two rats and reported as incidental.	
4.7 Other		
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	The study was carried out in accordance to FIFRA 885.3600. The rats received oral doses of acrolein in five days per week for 13 consecutive weeks. Three levels of acrolein in water, 0.05, 0.5, 5.0 mg/kg and a vehicle control group were used in this study.	
5.2 Results and discussion	Based on the results of observations made daily for clinical signs, weekly body weights, and food consumption estimates, haematology, clinical chemistry, and urinalysis, gross necropsy, organ weight data and histopathology, no significant toxic effects were found at the levels tested.	
5.3 Conclusion		
5.3.1 LO(A)EL	Not relevant, see NO(A)EL	
5.3.2 NO(A)EL	> 5.0mg/kg	
5.3.3 Other		
5.3.4 Reliability	1	
5.3.5 Deficiencies	No	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	04/05/06	
Materials and Methods	2.1 OECD guideline 408	
Results and discussion	As described by the Applicant.	
Conclusion	LO(A)EL: Not applicable NO(A)EL: > 5.0mg/kg	
Reliability	1	
Acceptability	acceptable	
Remarks		
	COMMENTS FROM ... *(specify)*	
Date	*Give date of comments submitted*	

Section A6.4.1 **Annex Point IIA6.4**	**Repeated dose toxicity** Subchronic oral toxicity of acrolein in rats	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Table A6_3-1. Results of repeated dose toxicity study

Parameter		Control		low dose		medium dose		high dose		dose-response +/-	
		m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
number of animals examined		10	10	10	10	10	10	10	10		
Mortality		0	0	1	0	0	0	0	0		
clinical signs*											
body weight											
food consumption											
clinical chemistry*											
haematology*											
urinalysis*											
Blood	±	1	0	2	0	0	0	0	3		
	+	0	0	1	0	0	1	0	0		
	2+	0	0	1	1	1	0	1	0		
Protein	±	7	8	5	6	2	3	9	7		
	+	9	11	6	8	8	15	6	10		
	2+	2	0	5	2	10	1	3	0		
Ketones	±	0	0	2	0	3	0	1	0		
	+	7	0	9	0	8	1	5	0		
	2+	0	0	1	0	4	0	0	0		
Organ x organ weight											
gross pathology											
microscopic pathology*											
Organ y											

[a] *number of animals affected/total number of animals*

Section A6.4.2 Annex Point IIA VI.6.4	Subchronic Dermal Toxicity Test	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	An acute dermal toxicity study (Section A6.1.2, Annex Point IIA6.2) and a 21-day repeated dose toxicity (dermal) study (Section A6.3.2, Annex Point IIA, VI.6.4.), have been carried out on the active substance. Also, the use pattern of acrolein would lead to minimal exposure from the dermal route. Therefore it is considered that a subchronic dermal toxicity test is not required and is not in the interests of animal welfare.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	05/10/07	
Evaluation of applicant's justification	Applicant's justification is acceptable	
Conclusion	Applicant's justification is acceptable	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A6.4.3/01 **Annex Point IIA 6.4**		**Repeated dose toxicity** Sub-chronic Inhalation in rats	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Kutzman, R.S. (1981) A Subchronic Inhalation Study of Fischer 344 Rats Exposed to 0, 0.4, 1.4, or 4.0 ppm Acrolein, Brookhaven National Laboratory, Study No. RD0148190.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Baker Petrolite	
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No guideline available	
2.2	**GLP**	No GLP was not compulsory at the time the study was performed	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	
3.1.1	Lot/Batch number		
3.1.2	Specification	As given in Section 2	
3.1.2.1	Description	See 3.1.2	
3.1.2.2	Purity	See 3.1.2	
3.1.2.3	Stability	See 3.1.2	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Fischer 344	
3.2.3	Source	Charles River laboratories, Inc., Kingston, New York	
3.2.4	Sex	Males and Females	
3.2.5	Age/weight at study initiation	Males: Approximately 200 – 250 g / 13 weeks Females: Approximately 150 – 160 g / 13 weeks	
3.2.6	Number of animals per group	52 males 52 females	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Inhalation	
3.3.1	Duration of treatment	62 days	
3.3.2	Frequency of exposure	5 days per week	

Section A6.4.3/01 Annex Point IIA 6.4		Repeated dose toxicity Sub-chronic Inhalation in rats		
3.3.3	Postexposure period	None		
3.3.4	Inhalation			X
3.3.4.1	Concentrations	Nominal concentration	0, 0.4, 1.4, 4.0 ppm	
		Analytical concentration		
3.3.4.2	Particle size	Not applicable		
3.3.4.3	Type or preparation of particles	Not applicable		
3.3.4.4	Type of exposure	Whole body		
3.3.4.5	Vehicle	Nitrogen		
3.3.4.6	Concentration in vehicle	1000 ppm		
3.3.4.7	Duration of exposure	6 hours		
3.3.4.8	Controls	Filtered air		
3.4	**Examinations**			
3.4.1	Observations			X
3.4.1.1	Clinical signs	No		
3.4.1.2	Mortality	Yes, daily		
3.4.2	Body weight	Yes, weekly		
3.4.3	Food consumption	No		
3.4.4	Water consumption	No		
3.4.5	Ophthalmoscopic examination	No		
3.4.6	Haematology	No		
3.4.7	Clinical Chemistry	No		
3.4.8	Urinalysis	No		
3.5	**Sacrifice and pathology**			
3.5.1	Organ Weights	Yes- selected animals Organs: Liver, kidneys, testes, spleen, brain, heart, lungs & trachea		
3.5.2	Gross and histopathology	Yes- selected animals All dose groups Organs: Lung, peribronchial lymph node, nasal turbinate, brain, kidney, liver, spleen, testes, heart.		
3.5.3	Other examinations	Respiratory tract		
3.5.4	Statistics			
3.6	**Further remarks**			

Section A6.4.3/01 **Annex Point IIA 6.4**		**Repeated dose toxicity** Sub-chronic Inhalation in rats	
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs		
4.1.2	Mortality	Refer to Table A6_3_1. Mortality among male rats exposed to 4.0 ppm acrolein reached 56% (32/57) while none of the female rats in the high dose group died.	
4.2	**Body weight gain**	Refer to Table A6_3_2. Both male and female rats in the 4.0 ppm acrolein chamber lost weight during the first 10 exposure days. Although all of the animals in each exposure group were weighed weekly, only data from the largest sub-groups entering the chambers on a single day were plotted and analysed (survivors only in the male 4.0 ppm subgroup).One way ANOVA indicated that the low and intermediate dose male animals were significantly ($p<0.0083$ by Bonferroni multiple comparison) heavier than the control males on the first day of exposure. This difference was not noted again throughout the exposure regime. Males exposed to 4.0 ppm acrolein gained weight at a significantly reduced rate. Among the female exposure groups the weights of the 0.4 and 4.0 ppm animals differed significantly at the first weighing. At all subsequent weighings the weight of the high dose group was significantly less than that of the other groups among which there were no significant differences.	
4.3	**Food consumption and compound intake**	No data	
4.4	**Opthalmoscopic examination**	No data	
4.5	**Blood analysis**		
4.5.1	Haematology	No data	
4.5.2	Clinical chemistry	No data	
4.5.3	Urinalysis	No data	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights		
4.6.2	Gross and histopathology		
4.7	**Other**		
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	There was no guideline available at the time the study was conducted. Fischer 344 rats were exposed to filtered air (control), 0.0, 0.4, 1.4 or 4.0 ppm acrolein for 6 hours/day, five days/week. Each animal was exposed for 62 consecutive days with exceptions only for weekends.	

Section A6.4.3/01 **Annex Point IIA 6.4**	**Repeated dose toxicity** Sub-chronic Inhalation in rats	
	The acrolein concentration of each chamber was automatically monitored for 5 minutes every half hour with a Miran infrared analyser.	
5.2 Results and discussion	Mortality was observed only in the 4.0 ppm chamber where 32 of 57 exposed males died; however none of the 8 exposed females died. Most of the mortality occurred within the first 10 days. Histological examination indicated that the animals died of acute bronchopneumonia. Both male and female rats in the 4.0 ppm acrolein chamber lost weight during the first 10 exposure days. Although all of the animals in each exposure group were weighed weekly, only data from the largest sub-groups entering the chambers on a single day were plotted and analysed (survivors only in the male 4.0 ppm subgroup). The surviving males and females exposed to 4.0 ppm acrolein gained weight at a significantly slower rate than control animals. The growth of both sexes in the 0.4 and 1.4 ppm groups was similar to that of their respective controls. In the 1.4 ppm exposure group, 3 of the 31 animals examined had lesions directly related to acrolein exposure. Extra respiratory organs appeared unaffected.	
5.3 Conclusion		
5.3.1 LO(A)EL	-	
5.3.2 NO(A)EL	1.4 ppm	
5.3.3 Other		
5.3.4 Reliability	2	
5.3.5 Deficiencies	The study was not conducted to a guideline or GLP.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*04/05/06*	
Materials and Methods	*3.3.4 Gaseous acrolein was tested.* *3.4.1 Respiratory physiology parameters were also measured in 24 rats /group: spontaneous breathing, heart rate, lung volumes, distribution of ventilation and flow volume dynamics.*	

Section A6.4.3/01 **Annex Point IIA 6.4**	**Repeated dose toxicity** Sub-chronic Inhalation in rats	
Results and discussion	*5.2 Restrictive lung lesion with supra-normal flow volume was observed in rats in the 0.4 ppm group. Animals in the 1.4 ppm group were similar to controls, although an increase in collagen was observed.* *Bronchiolar epithelial necrosis and sloughing, bronchiolar oedema with macrophages, focal pulmonary oedema were observed in the 4 ppm group. These findings were sometimes associated with oedema of the trachea and peri-bronchial lymph nodes, and acute rhinitis indicating upper respiratory tract effects. These were observed with varying incidence. A decrease in pulmonary function was observed (decreased flow volume, left ward shift of the quasi-static compliance curve and increase in lung volume) indicating obstructive lung disease. Increases were also observed in lung weight, lung connective tissue content, elastin and collagen.*	
Conclusion	*LO(A)EL:* *NO(A)EL:<0.4 ppm*	
Reliability	*2*	
Acceptability	*acceptable*	
Remarks		
	COMMENTS FROM ... *(specify)*	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Table A6_3-1. Results of repeated dose toxicity study

Parameter	**Control**		**low dose**		**medium dose**		**high dose**		**dose-response +/-**	
	m[a]	**f[a]**	**m[a]**	**f[a]**	**m[a]**	**f[a]**	**m[a]**	**f[a]**	**m**	**f**
number of animals examined										
Mortality							32/57	0/8	+	
clinical signs*										
body weight	↑	↑	↑	↑	↑	↑	↓	↓	+	+

Section A6.4.3/02 **Annex Point IIA 6.4**		**Repeated dose toxicity** Sub-chronic Inhalation in rats	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Kutzman, RS et al (1984) Selected responses of hypertension-sensitive and resistant rats to inhaled acrolein. Toxicology, 31: 53-65 Also Kutzman, RS et al (1986) The impact of inhaled acrolein on hypertension-sensitive and resistant rats. J Environ. Pathol. Toxicol. Oncol, 6(5-6) 97-108	
1.2	**Data protection**	No	
1.2.1	Data owner	Not applicable	
1.2.2	Criteria for data protection	Not applicable	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No guideline specified	
2.2	**GLP**	Not specified	
2.3	**Deviations**	Two strains of rat tested, one susceptible to salt-induced hypertension (DS) and the other resistant to salt-induced hypertension (DR)	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Gaseous acrolein purchased as a 1000 ppm mixture in Nitrogen	
3.1.1	Lot/Batch number	Not stated	
3.1.2	Specification	Not stated	
3.1.2.1	Description	Gaseous	
3.1.2.2	Purity	Not stated	
3.1.2.3	Stability	Not stated	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Dahl hypertension-resistant (DR) and Dahl hypertension-sensitive (DS) Developed from selective breeding of heterogenous population of Sprague-Dawley rats	
3.2.3	Source	Bred by Authors. Medical Department, Brookhaven National Laboratory, Upton, NY 11973, USA	
3.2.4	Sex	female	
3.2.5	Age/weight at study initiation	37 days old	
3.2.6	Number of animals per group	10 DS and 10 DR rats per exposure group	
3.2.7	Control animals	Yes, 10 DS and 10 DR rats.	

Section A6.4.3/02 **Annex Point IIA 6.4**		**Repeated dose toxicity** Sub-chronic Inhalation in rats		
3.3	**Administration/ Exposure**	Inhalation		
3.3.1	Duration of treatment	Maximum of 63 days		
3.3.2	Frequency of exposure	6 hours /day, 5 days per week		
3.3.3	Postexposure period	Seven days for half of control and surviving test animals. Other half of animals used for ancillary studies.		X
3.3.4	Inhalation			
3.3.4.1	Concentrations	Nominal concentration	0.4, 1.4, 4.0 ppm (The EU RA on Acrolein identified the dose levels as 0.9, 3.2, 9.2 mg acrolein vapour/m^3)	
		Analytical concentration		
3.3.4.2	Particle size	Not applicable		
3.3.4.3	Type or preparation of particles	Not applicable		
3.3.4.4	Type of exposure	Not specified		
3.3.4.5	Vehicle	Filtered air		
3.3.4.6	Concentration in vehicle	Not applicable		
3.3.4.7	Duration of exposure	6 hours		
3.3.4.8	Controls	Filtered air		
3.4	**Examinations**			
3.4.1	Observations	Behavioral testing of exploratory behaviour and locomotor activity performed five days after the final exposure.		
3.4.1.1	Clinical signs	Not specified		
3.4.1.2	Mortality	Yes, daily		
3.4.2	Body weight	Yes, after initial exposure and then weekly		
3.4.3	Food consumption	Not specified		
3.4.4	Water consumption	Not specified		
3.4.5	Ophthalmoscopic examination	Not specified		
3.4.6	Haematology	Blood serum (blood urea nitrogen, creatine, uric acid, calcium, phosphorus, alkaline phosphatase, serum glutamic oxalacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) and blood pressure		
3.4.7	Clinical Chemistry	Not specified		
3.4.8	Urinalysis	Not specified		

Section A6.4.3/02 **Annex Point IIA 6.4**		**Repeated dose toxicity** Sub-chronic Inhalation in rats	
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	Lung, heart, liver, kidneys, spleen and brain	
3.5.2	Gross and histopathology	Lungs, brain including olfactory bulbs and tissue extending 2 mm beyond medulla, heart, trachea, liver kidney, spleen and nasal turbinates.	
3.5.3	Other examinations	Not stated	
3.5.4	Statistics	Analysis of variance on behavioural measures	
3.6	**Further remarks**		
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	Not specified	
4.1.2	Mortality	Mortalities only occurred in the 4.0 ppm dose group. All DS animals exposed at the 4.0 ppm dose level died within eleven days of the initial exposure. Four out of 10 DR animals died during the 62 day exposure period.	
4.2	**Body weight gain**	Rats exposed to 0.4 and 1.4 ppm gained weight at rates comparable to controls. Surviving DR rats at 4.0 ppm rapidly lost approximately 15% of their starting weight. These animals remained at the reduced weight for 3 weeks after which they gained an average of 6.8 g per week. In the final 5 day post-exposure period before behavioural testing, the weight of these animals increased from an average of 161 g to 187 g.	
4.3	**Food consumption and compound intake**	Not specified	
4.4	**Opthalmoscopic examination**	Not specified	
4.5	**Blood analysis**		
4.5.1	Haematology	Blood pressure: No significant dose dependent differences in blood pressure were found. Although DS rats were fed a low-salt diet which generally maintains them normotensive, a significant blood pressure increase was observed when all DS exposure groups were compared to DR groups, independent of exposure to the test material. Serum chemistry: Several serum chemistry parameters in DR rats exposed to 4.0 ppm acrolein increased significantly from those of control DR rats. The most marked change was a 71% increase in alkaline phosphatase. Phosphorous levels were 53% higher and SGOT and SGPT were elevated 42% and 59% respectively. All other serum chemistry parameters were unchanged for the high dose DR rats. Exposure of DR and DS rats to 0.4 or 1.4 ppm acrolein did not significantly alter any of the serum chemistry variables.	

Section A6.4.3/02 **Annex Point IIA 6.4**		**Repeated dose toxicity** Sub-chronic Inhalation in rats	
4.5.2	Clinical chemistry	Not specified	
4.5.3	Urinalysis	Not specified	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	The lung to body weight ratio of DR rats exposed to 4.0 ppm acrolein was significantly greater than that of the air exposed DR group. The hearts, livers and brains of the 4.0 ppm DR rats were also proportionately heavier than those of controls. Rats exposed to 4.0 ppm acrolein gained significantly more weight than controls during the week between final exposure and sacrifice. Therefore, organ to body weight ratios for this exposure group may be lower than the organ to body weight ratios actually maintained during the exposure period.	
4.6.2	Gross and histopathology	Both DS and DR rats had subtle alteration of pulmonary parenchyma following exposure to 0.4 and 1.4 ppm acrolein. The lungs of 6/10 DS rats and 5/10 DR rats had a slight increase in peripheral lymphoid aggregates in comparison to controls. These aggregates, primarily composed of small uniformly shaped lymphocytes and an occasional larger phagocytic cell, were regularly found deep in pulmonary lobules. Conversely, bronchial asscociated lymphoid tissue appeared similar to that of controls. Collections of intraalveolar macrophages with foamy cytoplasm were present in 7/10 DS rats and 5/10 rats in the 0.4 and 1.4 ppm exposure groups. These macrophages, present in about 20% of the lobules, were often adjacent to acutely damaged terminal broncioles. In 8/10 DS and 5/10 DR rats, a mild terminal bronchiolar epithelial hyperplasia sometimes attended by a squamous metapasia and a nearly fibrocellular reaction was observed. The lungs of the 6 surviving DR high dose animals showed similar, but more severe pathologic changes than those of the 0.4 and 1.4 ppm groups. Although aggregates of peripheral-lymphoid tissue resembled those of the lower dose groups, a substantial increase was observed in the number of alveolar macrophage clusters and in the severity of alveolar wall mononuclear hypercellularity. Additionally, squanous metaplasia of tracheal epithelium, and hyperplastic/metaplastic terminal bronchiolar epithelial change was enhanced. A multifocal interstitial pneumonitis was present in 4/6 of these surviving high dose rats. Nasal turbinates from all surviving rats were essentially free of remarkable change, with mild acute inflammation found only on one section and excess mucus production on one other. The nasal tissues from dead rats were not available for examination. The non-pulmonary tissues from all groups failed to demonstrate microscopic change attributable to acrolein exposure. Examination of dead and moribund animals revealed pulmonary injury different than that observed in the rats.which survived the exposure regime. Although collections of macrophages were noted within alveoli and small distil airways, the predominant acute alteration involved necrosis of bronchial and bronchiolar epithelia with varying degrees of bronchopneumonia. From the medium sized bronchial branches distally to the terminal bronchioles, epithelial cells appeared swollen, necrotic and partially detached from subjacent membranes. Massive edema consistently attended all specimens with marked epithelial damage and in 70% of these, extravasation of red blood cells into alveolar spaces	

Section A6.4.3/02 **Annex Point IIA 6.4**		**Repeated dose toxicity** Sub-chronic Inhalation in rats	
		was noted. These alterations, probably the cause of death, were usually centro-lobular, but occasionally involved the alveoli of an entire lobe. A less severe but similar pattern of pulmonary injury was observed in the small number of DR animals that died during exposure to 4.0 ppm acrolein. Despite the necrotic change in small airways, the mucosal and epithelial surfaces of the trachea and main stem bronchi only showed varying degrees of squamous metaplasia.	
4.7	**Other**		X
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Ten hypertension-resistant and ten hypertension-sensitive Dahl rats were exposed to test concentrations of 0.4, 1.4 and 4.0 ppm gaseous acrolein via inhalation for 6 hours, 5 days a week for a maximum of 63 days. Control animals (10 of each type of rat) were exposed to filtered air. Body weights were recorded weekly, mortalities daily After exposure, the rats were left for 5 days and then tested for behavioural alterations. Histapathology was primarily performed on the lungs. Organ weight to body weight ratios were also recorded.	
5.2	**Results and discussion**	Comparison of DS and DR rats exposed to 0.4 and 1.4 ppm acrolein failed to reveal a dose-dependent phenomenon elucidating the differential mortality observed at 4.0 ppm acrolein. Behavioural changes dependent upon acrolein were not evident. The pattern of pulmonary pathology observed after acrolein exposure was dependent upon both the line of rat and concentration of inhaled acrolein. DS rats dosed at 4.0 ppm acrolein and found dead or moribund by the eleventh day had lungs exhibiting acute epithelial necrosis with concomitant oedema and hemorrhage. Rats dosed at 0.4 and 1.4 ppm acrolein showed proliferative morphologic lung changes. The uniform distribution of the change, centred at transitional terminal bronchiolar zone. Increased numbers of and clusters of macrophages were found in lungs of both types of rat particularly in the low and intermediate exposure groups. The phagocytic cell augmentation usually adjacent to damaged terminal bronchioles, probably represented a normal inflammatory response to acrolein induced cellular injury. Additionally, the increase in peripheral lymphoid aggregates in both types of rat appeared proportional to attendant tissue injury and to acrolein concentration.	
5.3	**Conclusion**	The NOAEL for the rat was 0.4 ppm (0.9 mg acrolein/m^3) based on lung changes	
5.3.1	LO(A)EL		
5.3.2	NO(A)EL	0.4 ppm (0.9 mg acrolein/m^3)	
5.3.3	Other		
5.3.4	Reliability	2	
5.3.5	Deficiencies		

Section A6.4.3/02 **Annex Point IIA 6.4**	**Repeated dose toxicity** Sub-chronic Inhalation in rats	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	09/05/06	
Materials and Methods	3.3.3 Behavioural studies were performed on all animals. Half of the animals in the control, 0.4 and 1.4 ppm dose groups and all the animals in the top dose group were sacrificed for necropsy. The remaining animals were used in ancillary studies.	
Results and discussion	4.7 Results for behavioural measures: No significant differences were found amongst any of the groups.	
Conclusion	LO(A)EL: NO(A)EL:<0.4 ppm due to dose reponse in severity of pathological lung changes.	
Reliability	2	
Acceptability	acceptable	
Remarks		
	COMMENTS FROM ... *(specify)*	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A6.4.3/03 **Annex Point IIA 6.4**		**Repeated dose toxicity** Sub-chronic Inhalation in rats	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Kutzman, RS et al (1985) Changes in rat lung structure and composition as a result of subchronic exposure to acrolein. Toxicology, 34: 139-151	
1.2	**Data protection**	No	
1.2.1	Data owner	Not applicable	
1.2.2	Criteria for data protection	Not applicable	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No guideline specified	
2.2	**GLP**	Not specified	
2.3	**Deviations**	Study designed to permit comparison of acrolein induced dose-related effects on lungs to historical changes in the same animals.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Gaseous acrolein purchased as a 1000 ppm mixture in Nitrogen	
3.1.1	Lot/Batch number	Not stated	
3.1.2	Specification	Not stated	
3.1.2.1	Description	Gaseous	
3.1.2.2	Purity	Not stated	
3.1.2.3	Stability	Not stated	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Fischer-344	
3.2.3	Source	Not specified	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Males: 238g Females 157g	
3.2.6	Number of animals per group	57 male, 8 female	
3.2.7	Control animals	Yes.	
3.3	**Administration/ Exposure**	Inhalation	
3.3.1	Duration of treatment	62 days	
3.3.2	Frequency of exposure	6 hours /day, 5 days per week	

Section A6.4.3/03 Annex Point IIA 6.4		**Repeated dose toxicity** Sub-chronic Inhalation in rats		
3.3.3	Postexposure period	6 days.		
3.3.4	Inhalation			
3.3.4.1	Concentrations	Nominal concentration	0.4, 1.4, 4.0 ppm (The EU RA on Acrolein identified the dose levels as 0.9, 3.2, 9.2 mg acrolein vapour/m^3)	
		Analytical concentration		
3.3.4.2	Particle size	Not applicable		
3.3.4.3	Type or preparation of particles	Not applicable		
3.3.4.4	Type of exposure	Not specified		
3.3.4.5	Vehicle	Filtered air		
3.3.4.6	Concentration in vehicle	Not applicable		
3.3.4.7	Duration of exposure	6 hours		
3.3.4.8	Controls	Filtered air		
3.4	**Examinations**			
3.4.1	Observations	Pulmonary testing performed before sacrifice.		
3.4.1.1	Clinical signs	Not specified		
3.4.1.2	Mortality	Yes, daily		
3.4.2	Body weight	Yes, after initial exposure and then weekly		
3.4.3	Food consumption	Not specified		
3.4.4	Water consumption	Not specified		
3.4.5	Ophthalmoscopic examination	Not specified		
3.4.6	Haematology	Not specified		
3.4.7	Clinical Chemistry	Not specified		
3.4.8	Urinalysis	Not specified		
3.5	**Sacrifice and pathology**			
3.5.1	Organ Weights	Lungs and trachea, heart, liver, kidneys, spleen, testis and brain from males only		
3.5.2	Gross and histopathology	Lungs only. Lung composition: Concentration of hydroxyproline (collagen content), total elastin and protein. Histology:		

Section A6.4.3/03 **Annex Point IIA 6.4**		**Repeated dose toxicity** Sub-chronic Inhalation in rats	
		Alveolitis, type II cell hyperplasia, increasing numbers of alveolar macrophages, bronchiolar epithelial necrosis and sloughing, haemorrhage, bronchiolar oedema and macrophages and chronic pleuritis.	
3.5.3	Other examinations	Not stated	
3.5.4	Statistics	One-way analysis of variance (ANOVA) for single parameter means amongst exposure groups. Students t-test for all possible combinations of group mean pairs to investigate source of differences. Bonferroni method for adjustment for problem of multiple comparisons. Kruskal_Wallis test for non-parametric data for examination of histopathology data for differences among the groups. Non-parametric multiple comparison technique for comparison of possible paired combinations of exposure groups for differences.	
3.6	**Further remarks**		
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	Not specified	
4.1.2	Mortality	Mortality among male rats exposed to 4.0 ppm acrolein reached 56% (32/57) while none of the female rats in this chamber died. The greatest mortality occurred from the eighth through the tenth day of exposure. Animals were introduced into the exposure chambers on 4 of the 5 exposure days of the week to accommodate the endpoint assessment schedule; therefore, the high mortality observed on days 8 through 10 was not a reflection of 3 – 5 consecutive days of acrolein insult during the second week of exposure.	
4.2	**Body weight gain**	Both male and female rats in the 4.0 ppm acrolein chamber lost weight during the first 10 exposure days, after which both of these groups began to gain weight. At all time points following the initial exposure day the mean weights of the 4.0 ppm groups were significantly less than those of the control and other exposure groups. The growth rates of animals in the 0.4 and 1.4 ppm chambers were not different from those of control rats. During the post-exposure period, groups of male and female rats removed from the 4.0 ppm gained an average of 35.1g and 24.0 g respectively, over the 6-day post-exposure period. Among control, 0.4 and 1.4 ppm exposure groups, the post-exposure weight gain was less marked, but very similar.	
4.3	**Food consumption and compound intake**	Not specified	
4.4	**Opthalmoscopic examination**	Not specified	
4.5	**Blood analysis**		

Section A6.4.3/03 Annex Point IIA 6.4		Repeated dose toxicity Sub-chronic Inhalation in rats	
4.5.1	Haematology	Not specified	
4.5.2	Clinical chemistry	Not specified	
4.5.3	Urinalysis	Not specified	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	The organ-to-body weight ratios of all of the organs, with the exception of the liver and spleen, were significantly greater in the somewhat emaciated 4.0 ppm animals than in other groups. The most marked increase in organ-to body weight ratio was observed for the lungs of the 4.0 ppm exposure group. However, the lung-to-body weight ratio was not increased in the animals exposed to 0.4 and 1.4 ppm acrolein.	
4.6.2	Gross and histopathology	Lung composition: The lungs from animals in the 4.0 ppm chamber were significantly heavier then those of the other exposure groups. While the lung weights and total dry weights of the lungs increased 33% and 21% respectively, the percent dry weight decreased 1.5%, indicating a slight but significant increase in water content. Exposure to 0.4 or 1.4 ppm acrolein did not result in changes in these parameters. The total DNA and protein contents of the lungs from male rats exposed to 4.0 ppm acrolein were 117% and 120% of the control lungs, respectively. The increased lung weight coincident with proportional increases in total dry weight resulted in loss of these differences when DNA and protein contents were expressed in terms of dry weight. The elastin content of the lungs from animals exposed to 0.4 or 1.4 ppm acrolein was not changed from that of controls. However, exposure to 4.0 ppm acrolein resulted in an elastin content which was 174% of the control value. Although the increase observed in the amount of hydroxyproline, an index of collagen, was not as great as that observed for elastin in the 4.0 ppm group, total collagen content was also increased in the 1.4 ppm group. Based on dry weight, the collagen concentrations in lungs from the 1.4 and 4.0 ppm animals were 113% and 137% respectively of the control concentration. Histological findings: Lungs from animals found dead or moribund in the 4.0 ppm chamber displayed severe acute bronchopneumonia, however, several areas of the lungs appeared unaffected. There was focal alveolar oedema with sloughed cells in the bronchi and bronchioles. Many of the airways were actually plugged which could have resulted in anoxia and death, even though there were healthy areas in the lungs. In addition, to the pulmonary changes, there was tracheal oedema with erosion of the mucosal epithelium. The lungs from control rats sacrificed post-exposure displayed minimal to slight proliferations of lymphoid cells associated with a low-grade chronic murine pneumonia. The presence of a slight acute or subacute alveolitis in some of these animals suggested a recent bacterial infection. These changes were not severe and are mentioned for the purpose of baseline pulmonary pathology. The 0.4 ppm exposure group did not display pulmonary lesions attributable to acrolein exposure. Lungs from 3 of the 1.4 ppm rats examined appeared to have exposure related pulmonary lesions which consisted of bronchiolar epithelial necrosis and sloughed cells lying free	

Section A6.4.3/03 **Annex Point IIA 6.4**	**Repeated dose toxicity** Sub-chronic Inhalation in rats	
	in the lumen. Exposure to this concentration of acrolein resulted in increased numbers of alveolar macrophages and it enhanced the degree of type II cell hyperplasia. Also, changes associated with chronic murine pneumonia or a focal subacute alveolitis appeared somewhat enhanced by this acrolein exposure. Lungs from the 4.0 ppm exposure group exhibited the following lesions: bronchiolar epithelial necrosis and sloughing, bronchiolar mucopurulent plugs with macrophages and focal pneumonitis. The numbers of alveolar macrophages also appeared increased. Oedema in the trachea and peribronchial lymph nodes also appeared to be exposure related in this group as did acute rhinitis. The severity of the lung lesions was highly variable and 3 of the 9 animals examined histologically did not demonstrate structural pulmonary effects. The severity of pulmonary lesions observed in the left lungs of these animals was subjectively scored for severity. Although mild histologic changes were observed in the control animals, a dose-related increase in pathologic change was clearly evident in the acrolein treated groups. Also, the broad range of intragroup variability, particularly in response to 1.4 and 4.0 ppm acrolein was apparent. Many of the animals in the 1.4 ppm group had scores which overlapped those of the controls, while 3 of the 9 4.0 ppm animals showed no histologic damage.	
4.7 Other		
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	Fischer-344 rats (57 males and 8 females per dose group) were dosed with 0.4, 1.4 or 4.0 ppm gaseous acrolein by inhalation to study the dose related effects of the test material material on the lungs in comparison with historical changes in the same animals. A control group dosed with filtered air was run along side the dose groups. Animals were dosed for 6 hours a day, 5 days a week for 62 days. Examinations of body weight, organ weights and pulmonary tests including histopathology on lungs were performed. Mortalities were recorded daily.	
5.2 Results and discussion	The mortality observed among male rats in the 4.0 ppm chamber predominantly occurred during the first three weeks of exposure. No female rats exposed to 4.0 ppm acrolein died although they rapidly lost weight and remained at less than their starting weights throughout the exposure period. After the initial period of weight loss and mortalities, animals in the 4.0 ppm dose group began to gain weight at a rate similar to that of controls. For the 4.0 ppm dose group, organ-to-body weight ratios may have changed from those maintained during the treatment period due to the weight gain over the final post-exposure period. The greater absolute lung weight of animals exposed at 4.0 ppm reflected an apparent increased cellularity. Although total DNA and protein increased in the high dose group, the amount of these lung constituents per unit dry weight remained constant. This increased tissue mass may, in part, be accounted for by the type II cell hyperplasia and the infiltration of macrophages. However, it is unlikely that these 2 cell types account for the 33% weight increase in the 4.0 ppm lungs over those of controls. Increased numbers of pulmonary macrophages were found in the	

Section A6.4.3/03 **Annex Point IIA 6.4**	**Repeated dose toxicity** Sub-chronic Inhalation in rats	
	bronchiolar regions of 4.0 ppm animals. However, these cells may have accumulated in damaged bronchioles during the post-exposure period. The absence of overt pathological changes in several of the animals was unexpected considering the mortalities at this dose level. There was also marked intragroup variability in the 1.4 ppm dose group. The reasons for this are unclear. Histological examination did not provide a clear indication of the location of the increased connective tissue present in the lungs of the 4.0 ppm exposed animals. This increased collagen and elastin concentration may have been resultant to the bronchiolar epithelial lesion observed in this group since lesions suggestive of interstitial or focal fibrosis were not apparent.	
5.3 Conclusion	The NOAEL for the rat was 1.4 ppm (3.2 mg acrolein/m^3) based on lung changes	
5.3.1 LO(A)EL		
5.3.2 NO(A)EL	1.4 ppm (3.2 mg acrolein/m^3)	
5.3.3 Other		
5.3.4 Reliability	2	
5.3.5 Deficiencies		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*08/05/06*	
Materials and Methods	*This data is from the study by Kutzman 1981: A sub-chronic Inhalation Study of Fischer 344 rats exposed to 0, 0.4, 1.4 4.0 ppm acrolein. The full study is presented in A6-4-3(01). Details regarding lung physiology part of the initial study are summarised in A6-4-3(04) Costa 1986: Altered lung function and structure in the rat after subchronic exposure to acrolein.*	
Results and discussion		
Conclusion	*LO(A)EL: 1.4 ppm for lung structure effects* *NO(A)EL: 0.4 ppm for lung structure effects*	
Reliability	*2*	
Acceptability	*acceptable*	
Remarks		
	COMMENTS FROM ... *(specify)*	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	

Section A6.4.3/03 **Annex Point IIA 6.4**	**Repeated dose toxicity** Sub-chronic Inhalation in rats	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A6.4.3/04 **Annex Point IIA 6.4**		**Repeated dose toxicity** Sub-chronic Inhalation in rats	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Costa DL et al (1986) Altered lung function and structure in the rat after subchronic exposure to acrolein. Am. Rev. Respir. Dis. 133: 286-291	
1.2	**Data protection**	No	
1.2.1	Data owner	Not applicable	
1.2.2	Criteria for data protection	Not applicable	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No guideline specified	
2.2	**GLP**	Not specified	
2.3	**Deviations**	Study designed to permit comparison of acrolein induced dose-related effects on lungs to historical changes in the same animals. See IIIA 6.4.3(03)	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Gaseous acrolein purchased as a 0.1% in Nitrogen	
3.1.1	Lot/Batch number	Not stated	
3.1.2	Specification	Not stated	
3.1.2.1	Description	Gaseous	
3.1.2.2	Purity	Not stated	
3.1.2.3	Stability	Not stated	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Fischer-344	
3.2.3	Source	Charles River Laboratories Inc. Kingston, NY, USA	
3.2.4	Sex	Male	
3.2.5	Age/weight at study initiation	Males: 239g – 245 g	
3.2.6	Number of animals per group	24	
3.2.7	Control animals	Yes.	
3.3	**Administration/ Exposure**	Inhalation	
3.3.1	Duration of treatment	62 days	
3.3.2	Frequency of exposure	6 hours /day, 5 days per week	

Section A6.4.3/04 Annex Point IIA 6.4		Repeated dose toxicity Sub-chronic Inhalation in rats		
3.3.3	Postexposure period	6 days.		
3.3.4	Inhalation			
3.3.4.1	Concentrations	Nominal concentration	0.4, 1.4, 4.0 ppm (The EU RA on Acrolein identified the dose levels as 0.9, 3.2, 9.2 mg acrolein vapour/m^3)	
		Analytical concentration	0.4, 1.4, 4.0 ppm	
3.3.4.2	Particle size	Not applicable		
3.3.4.3	Type or preparation of particles	Not applicable		
3.3.4.4	Type of exposure	Not specified		
3.3.4.5	Vehicle	Filtered air		
3.3.4.6	Concentration in vehicle	Not applicable		
3.3.4.7	Duration of exposure	6 hours		
3.3.4.8	Controls	Filtered air		
3.4	**Examinations**			
3.4.1	Observations	Pulmonary testing – physiologic measurements and pulmonary function		
3.4.1.1	Clinical signs	Not specified		
3.4.1.2	Mortality	Yes, daily (See IIIA 6.4.3(03))		
3.4.2	Body weight	Not specified (See IIIA 6.4.3(03))		
3.4.3	Food consumption	Not specified (See IIIA 6.4.3(03))		
3.4.4	Water consumption	Not specified		
3.4.5	Ophthalmoscopic examination	Not specified		
3.4.6	Haematology	Not specified		
3.4.7	Clinical Chemistry	Not specified		
3.4.8	Urinalysis	Not specified		
3.5	**Sacrifice and pathology**			
3.5.1	Organ Weights	Lungs only (See IIIA 6.4.3(03))		
3.5.2	Gross and histopathology	Lungs only. Morphologic appraisal and morphometric analysis presented in paper. See IIIA 6.4.3(03) for full postmortem results.		
3.5.3	Other examinations	Not stated		
3.5.4	Statistics	One-way analysis of variance (ANOVA) for comparison means of each variable amongst exposure groups. When ANOVA indicated a		

Section A6.4.3/04 **Annex Point IIA 6.4**		**Repeated dose toxicity** Sub-chronic Inhalation in rats	
		significant difference ($p < 0.05$), Duncan's multiple range test was applied to identify between group differences. Correlations between various functional and compositional variables were calculated using Pearson's product-moment correlation.	
3.6	**Further remarks**		
		4 RESULTS AND DISCUSSION	
4.1	**Observations**	Tidal breathing: Only rats exposed to 4.0 ppm showed significant changes in tidal volume, breathing frequency and pulmonary resistance compared to controls. Lung volumes: A significant increase compared to controls was noted in the 4.0 ppm dose group for total lung capacity, residual volume, functional residual capacity, vital capacity and inspiratory capacity. The disproportionate increase in residual volume resulted in a relative decrease in the vital capacity/total lung capacity ratio. Distribution of ventilation: Rats in the 4.0 ppm dose group has uneven distribution of ventilation with lung compartments emptying faster than those of controls. Flow volume dynamics: Acrolein treatment related changes in flow volumes were noted in the 0.4 and 4.0 ppm dose groups. At 0.4 ppm the flows were elevated above controls whereas at 4.0 ppm flows were depressed.	
4.1.1	Clinical signs	Not specified	
4.1.2	Mortality	There was 65% mortality in the 4.0 ppm dose group. There were no other mortalities. (See IIIA 6.4.3(03))	
4.2	**Body weight gain**	Surviving rats in the 4.0 ppm dose group appeared to stablise weight loss at 26% below control for the remainder of the exposure. (See IIIA 6.4.3(03))	
4.3	**Food consumption and compound intake**	Not specified	
4.4	**Opthalmoscopic examination**	Not specified	
4.5	**Blood analysis**		
4.5.1	Haematology	Not specified	
4.5.2	Clinical chemistry	Not specified	
4.5.3	Urinalysis	Not specified	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	(See IIIA 6.4.3(03))	
4.6.2	Gross and	Morphologic changes in lung tissues caused by acrolein generally were	

Section A6.4.3/04 Annex Point IIA 6.4	Repeated dose toxicity Sub-chronic Inhalation in rats	
histopathology	greater at the higher exposure levels, with severe peribronchiolar and bronchiolar damage apparent in the rats surviving the 4.0 ppm dose level. Similar exposure-related lesions, i.e. alveolitis, haemorrhage, end-airway epithelial sloughing, oedema and type II cell hyperplasia were observed in only 10% of rats dosed at 1.4 ppm and none of the rats dosed at 0.4 ppm. Rats exposed to 0.4 ppm had slightly reduced alveolar mean linear intercepts, whereas rats in the 4.0 ppm dose group exhibited a 13% increase. Internal surface areas of the lung were increased in all treatment groups. Parenchymal tissue density was altered in the 0.4 ppm dose group only. Alveolar ductal space was reduced 14% at this dose level, however, it was unchanged from control values in other exposure groups. There were several significant associations between functional and compositional characteristics in controls. Elastin concentration was associated with functional residual capacity, hydroxyproline content with breathing frequency and protein with peak expiratory flow, forced expiratory flow and volume expired. These associations were not evident in the treated groups. In the 4.0 ppm dose group elastin was correlated to forced expiratory flow, upstream airway resistence and to residual volume, hydroxyproline to residual volume and dynamic compliance and lung weight to residual volume. (See IIIA 6.4.3(03))	
4.7 Other		
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	Male Fischer-344 rats (24 per dose group) were dosed with 0.4, 1.4 or 4.0 ppm gaseous acrolein by inhalation to study the dose related effects of the test material on the exposure (dose) dependency of attributable alterations in rat lung function and the presence of discernible structure-function relationships within and among the exposure groups. A control group dosed with filtered air was run along side the dose groups. Animals were dosed for 6 hours a day, 5 days a week for 62 days. Examinations of lung morphology and morphometric analysis were performed after 62 days exposure. Other examinations were performed and discussed in a separate paper, see IIIA 6.4.3(03)	
5.2 Results and discussion	Substantial decrement in pulmonary function was observed in animals dosed at 4.0 ppm. The results of examinations indicated the presence of obstructive lesions in both small and large airways. Findings suggested that independent regions of the deep lung, including small airways, were sufficiently injured to impair their ventilation. The mechanism or relative specificity of apparent acrolein-stimulated lung growth is unclear. Rats exposed to 0.4 ppm acrolein exhibited "supernormal" maximal airflows, suggesting greater patency or stability of interdependent flow limiting airways. It is suggested that prolonged exposure to acrolein at low concentrations may have imparted macromolecular rearrangement or bonding within the supporting infrastructure of flaccid airway tissues without ostensible tissue changes. Acrolein under the exposure regime studies seems to have produced	

Section A6.4.3/04 **Annex Point IIA 6.4**	**Repeated dose toxicity** Sub-chronic Inhalation in rats	
	distinct functional lesions that were expressed in a contradictory or compensatory manner, depending on the exposure level. The extremes of the functional lesions were observed at 0.4 and 4.0 ppm, whereas these effects were essentially cancelled in the apparently normal 1.4 ppm group.	
5.3 Conclusion	The NOAEL for the rat was 0.4 ppm (0.9 mg acrolein/m^3) based on lung changes	
5.3.1 LO(A)EL		
5.3.2 NO(A)EL	0.4 ppm (0.9 mg acrolein/m^3)	
5.3.3 Other		
5.3.4 Reliability	2	
5.3.5 Deficiencies		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	08/05/06	
Materials and Methods	This data is from the study by Kutzman 1981: A sub-chronic Inhalation Study of Fischer 344 rats exposed to 0, 0.4, 1.4 4.0 ppm acrolein. The full study is presented in A6-4-3(01). Details regarding lung structure of the initial study are summarised in A6-4-3(03) Kutzman 1985: Changes in rat lung structure and composition as a result of subchronic exposure to acrolein.	
Results and discussion		
Conclusion	LO(A)EL: NO(A)EL: <0.4 ppm for physiological parameters	
Reliability	2	
Acceptability	acceptable	
Remarks		
	COMMENTS FROM ... *(specify)*	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A6.4.3/06 Annex Point IIA 6.4		**Repeated dose toxicity** Sub-chronic Inhalation in rats	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Feron, VJ et al (1978) Repeated exposure to acrolein vapour: subacute studies in hamsters, rats and rabbits. Toxicology 9: 47 - 57	
1.2	**Data protection**	No	
1.2.1	Data owner	Not applicable	
1.2.2	Criteria for data protection	Not applicable	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No guideline specified	
2.2	**GLP**	No GLP was not compulsory at the time the study was performed	
2.3	**Deviations**	Full examinations not performed on rat although study identified as most susceptible species	
		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	Not applicable	
3.1.2	Specification	Synthesised by Central Institute for Nutrtion and Food Research TNO, Zeist, The Netherlands	
3.1.2.1	Description	Liquid evaporated in dry nitrogen gas	
3.1.2.2	Purity	Not specified	
3.1.2.3	Stability	Not specified	
3.2	**Test Animals**		
3.2.1	Species	Rat, hamster, rabbit	
3.2.2	Strain	SPF, Wistar rat Syrian golden hamster Dutch rabbits	
3.2.3	Source	Rat and hamster: Central Institute for the Breeding of Laboratory Animals TNO, Zeist, The Netherlands Rabbit: Broekman Insitute B.V., Helmond, The Netherlands	
3.2.4	Sex	Males and Females	
3.2.5	Age/weight at study initiation	Rat: 98 – 124 g, 7 weeks old Hamster: 88 – 124 g, 10 weeks old Rabbit: 0.66 – 1.22 kg, 6 – 9 weeks old	
3.2.6	Number of animals per group	Rat: 12 per dose level Hamster: 20 per dose level Rabbits: 4 per dose level	
3.2.7	Control animals	Yes, as dose group	

Section A6.4.3/06 Annex Point IIA 6.4		**Repeated dose toxicity** Sub-chronic Inhalation in rats		
3.3	**Administration/ Exposure**	Inhalation		
3.3.1	Duration of treatment	13 weeks		
3.3.2	Frequency of exposure	5 days per week		
3.3.3	Postexposure period	No		
3.3.4	Inhalation			
3.3.4.1	Concentrations	Nominal concentration	0.4, 1.4, 4.9 ppm (0.9, 3.3, 11.5 mg acrolein vapour/m^3)	
		Analytical concentration	0.9, 3.3, 11.5 mg acrolein vapour/m^3	
3.3.4.2	Particle size	Not applicable		
3.3.4.3	Type or preparation of particles	Not applicable		
3.3.4.4	Type of exposure	Not specified		
3.3.4.5	Vehicle	Conditioned air		
3.3.4.6	Concentration in vehicle	Not specified		
3.3.4.7	Duration of exposure	6 hours		
3.3.4.8	Controls	Conditioned air		
3.4	**Examinations**			
3.4.1	Observations			
3.4.1.1	Clinical signs	General observations on appearance and behaviour.		
3.4.1.2	Mortality	Yes, daily		
3.4.2	Body weight	Once a week		
3.4.3	Food consumption	For rats and rabbits: recorded over 1 week periods during the first 4 weeks		
3.4.4	Water consumption	Not stated		
3.4.5	Ophthalmoscopic examination	Not stated		
3.4.6	Haematology	In week 12: Rabbit and hamster: haemoglobin content, haematocrit value, erythrocyte counts and total and differential leucocyte counts. Rat: Haemoglobin content and haematocrit value		
3.4.7	Clinical Chemistry	After exposure: Rabbits and hamsters only – serum activities of glutamic-oxaloacetic and glutamic-pyruvic transaminases and of alkaline phosphatase		

Section A6.4.3/06 **Annex Point IIA 6.4**		**Repeated dose toxicity** Sub-chronic Inhalation in rats	
3.4.8	Urinalysis	In week 12: Pooled urine samples for each species: pH, glucose, protein, ketones, occult blood and constituents of the sediment	
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	All animals except for mortalities during exposure period: Heart, liver, kidneys spleen, brain, gonads, lungs, thymus (except rabbits) and adrenals.	
3.5.2	Gross and histopathology	All animals: Tissues of heart, liver, kidneys spleen, brain, gonads, lungs, thymus (except rabbits), adrenals, gastrointestinal tract, thyroid, aorta, uterus, pancreas, mesenteric lymph nodes, skin, skeletal muscle, urinary bladder, salivary glands and head (after removal of the skin, brain and lower jaw).	
3.5.3	Other examinations		
3.5.4	Statistics	Student's t-test for the changes in body weights and organ-to-body weight ratios. Wilcoxon for haematological and biochemical values	
3.6	**Further remarks**		
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	During the exposures all animals treated with 4.9 ppm acrolein kept their eyes closed most of the time. In addition, the hamsters of this group salivated and had nasal discharge, the rabbits sneezed and occasionally breathed with difficulty and the rats had bristling hair. Hamsters and rats of the intermediate dose group usually fell asleep after a few minutes of hyperactivity at the start of each exposure, but they remained a little rest;less while sleeping. In rabbits of the mid-dose group, some sneezing was occasionally observed. At the lowest dose level no abnormal behaviour was seen in any of the animal species.	
4.1.2	Mortality	Three male and three female rats of the top dose group died in the first 4 weeks of the test period. No further deaths occurred in rats. In week 12, one male hamster of the highest concentration group had to be killed in moribund condition due to renal failure caused by extensive amyloid deposits in the kidneys, which were also found in several other organs. The conditions of this hamster was not considered related to treatment, since none of the other hamsters showed similar changes. Two rabbits died, 1 female of the control group in week 2 and 1 male of the 0.4 ppm group in week 12. The control animal was emaciated and showed upon microscopy, focal vacuolisation in the cerebral cortex, pharynigitis and necrotic epithelium in the large intestines.	
4.2	**Body weight gain**	In each of the animal species growth was clearly depressed at the highest exposure level Decreases for rats were 2/8%, 15/13% and 38/25% for males/females in 0.4, 1.4 and 4.9 ppm dose groups,	

Section A6.4.3/06 Annex Point IIA 6.4		**Repeated dose toxicity** Sub-chronic Inhalation in rats	
		respectively. Decrease in bodyweight of 12% for the combined sexes in rabbits and 20/31% for male/female hamsters. At the intermediate level decreased body weight gain was found in rats and rabbits (a decrease in the body weight of rabbits was also observed in the mid dose group (11%)., but not in hamsters. In the low dose group a slight but consistent growth retardation occurred in rats only, the differences with the controls, however, being statistically insignificant for both males and females.	
4.3	**Food consumption and compound intake**	Food intake in rats and rabbits appeared to be diminished in mid- and top-dose animals of both species.	
4.4	**Opthalmoscopic examination**		
4.5	**Blood analysis**		
4.5.1	Haematology	Haematologocal values in rats and rabbits were not affected by acrolein, but in hamsters, females of the top-dose group showed statistically significant increases in the number of erythrocytes, packed cell volume, haemoglobin content and the number of lymphocytes accompanied by a decrease in the number of neutrophilic leucocytes.	
4.5.2	Clinical chemistry	All serum enzyme activities were within normal ranges and no statistically significant differences occurred between the various test groups and the control group.	
4.5.3	Urinalysis	At the highest exposure level the urinary sediment appeared to contain a slightly increased amount of amorphous material in each of the 3 animal species examined. In hamsters and rats this increase was accompanied by a slight decrease in the number of urinary crystals.	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	Changes in organ-to-body weight ratios that could be attributed to treatment were only found at the highest exposure level. The changes comprised increases in the relative weights of the lungs in each of the animal species, of the heart and kidneys in hamsters and rats, and of the adrenals in rats. The increases in the relative weights of the brain and gonads found in hamsters and rats, and the decreases of the relative thymus weights observed only in rats, were considered reflections of reduced body weight gain rather than indications of an effect of acrolein on these organs.	
4.6.2	Gross and histopathology	Gross autopsy findings were essentially negative in hamsters and rabbits. The lungs of several rats of the highest concentration group that died showed patchy consolidation, haemorrhages and collapsed dark-reddish-purple areas. In addition, one male rat of this group that survived the experimental period had a chronic pleuritis as indicated by the presence of fibrous adhesions. Histopathological changes that could be attributed to acrolein exposure were observed only in the respiratory tract. At the highest exposure level marked changes were found in the epithelial lining of nasal cavity in each animal species. Necrotising rhinitis was occasionally seen in the dorsomedial part of the nasomaxillary region. The normal epithelium	

Section A6.4.3/06 **Annex Point IIA 6.4**	**Repeated dose toxicity** Sub-chronic Inhalation in rats	
	was partly replaced by stratified squamous epithelium occasionally showing keratinisation. Neutraphilic infiltration of the mucosa was invariably observed, whereas substantial neutrophilic exudation in the lumen was seen only in a few animals. At the intermediate level squamous metaplasia and neutrophilic infiltration of the mucosa occurred in rats, whereas in hamsters of this group minimal inflammatory alterations were seen in the nasal cavity. One male rat of the low-dose group showed metaplastic and inflammatory changes in this part of the respiratory tract. The nasal cavity of rabbits of the low and mid dose groups and of hamsters of the low-dose group was indistinguishable from that of the controls. The laryngeal epithelium, especially that covering the vocal cords and the region caudal to the vocal cords, was slightly thickened in a few female hamsters of thetop-dose group. In most of the rats of this group it was definitely metaplastic as indicated by the occurrence of keratinised stratified squamous epithelium. Effects of acrolein on the trachea were exclusively seen at the hioghest exposure level, and occurred in each of the animal species. In rabbits the tracheal epithelium looked hyperplastic and the number of mucus-producing cells was increased. In hamsters focal hyper- and metaplasia of the tracheal epithelium was observed in a few males and nearly all females of the top dose group. Stratification of the epithelium was a common finding, but keratinisation could not be demonstrated unequivocally. At the top dose the trachea of rats was severely damaged, whereas at lower exposure levels the epithelial lining was within normal limits. In addition to fairly extensive areas lined by metaplastic epithelium, nodules of granulation tissues covered by undifferentiated epithelium protruded into the lumen. Treatment-related histopathological changes in the bronchi and lungs were found in rats and rabbits of the top dose group, whereas in hamsters these portions of the respiratory tract were unaffected by acrolein exposure and resembled controls. The changes found in rats consisted of haemorrhages and perivascular and alveolar oedema (only in rats that died), focal broncho-pneumonia, bronchitis, bronchiolitis, hyper- and metaplasia of the bronchial and bronchiolar epithelium, increased numbers of mucus-producing cells in the bronchioles, accumulations of alveolar macrophages, and focal interstitial pneumonitis. There were considerable differences in degree of the lesions between the individual rats. In rabbits the bronchopulmonary lesions observed were similar in type to those encountered in rats, but were on average slightly less severe.	
4.7 Other		
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	The sub-chronic inhalation toxicity of acrolein of unknown purity was determined in rats, rabbits and hamsters of both sexes, 6 hours a day, 5 days a week for a period of 13 weeks. The doses tested were 0.4, 1.4 and 4.9 ppm (0.9, 3.3 and 11.5 mg acrolein vapour/m^3). The number of animals tested were rat: 12 per dose level, hamster: 20 per dose level, rabbit: 4 per dose level. Mortalities, signs of toxicity, food consumption and bodyweight measurements were made during the exposure period. Haematology and urinalysis were performed in week 12 of the exposure.	

Section A6.4.3/06 **Annex Point IIA 6.4**	**Repeated dose toxicity** Sub-chronic Inhalation in rats	
	Autopsy and gross histopathology on all animals was performed upon termination of exposure. All mortalities were autopsied.	
5.2 Results and discussion	Repeated exposure to an atmosphere containing up to 4.9 ppm acrolein vapour induced marked changes in each of the animal species examined. Between rats and hamsters, rats were most severely affected as appeared from mortality, severe growth retardation, increased adrenal weights and marked pathological changes in the different portions of the respiratory tract. In addition, rats of the low-dose group showed slight treatment related changes, whereas in both hamsters and rabbits 0.4 ppm was found to be a NOEL level. Unlike rats and rabbits, hamsters did not exhibit histopathological alterations in the broncho and lungs that could be attributed to the acrolein exposure. This may indicate that the lower respiratory tract of hamsters is less susceptible to acrolein than that of rats and rabbits. Another explanation may be that in hamsters a smaller part of the acrolein vapour reaches the peripheral segments of the respiratory tract than in the other animal species examined. Slight growth depression and minimal histopathological changes in the nasal cavity were still visible in rats exposed to 0.4 ppm. The effects of acrolein on the respiratory tract are summarised as destruction and hyper- and metaplasia of the lining epithelium accompanied by acute and subacute inflammatory alterations.	
5.3 Conclusion	NOAEL for the rat was < 0.4 ppm (< 0.9 mg acrolein.m^3)	
5.3.1 LO(A)EL		
5.3.2 NO(A)EL	< 0.4 ppm (< 0.9 mg acrolein m^3)	
5.3.3 Other		
5.3.4 Reliability	2	
5.3.5 Deficiencies	Full examinations not performed on rat although study identified as most susceptible species	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	09/05/06	
Materials and Methods	As described by the Applicant.	
Results and discussion	As described by the Applicant.	
Conclusion	LO(A)EL: NO(A)EL: rat <0.4 ppm rabbit, hamster 0.4ppm	
Reliability	2	
Acceptability	acceptable	
Remarks		

Section A6.4.3/06 **Annex Point IIA 6.4**	**Repeated dose toxicity** Sub-chronic Inhalation in rats	
	COMMENTS FROM ... *(specify)*	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Table A6_3-1. Summary results of repeated dose toxicity study in rat

Parameter	0.4 ppm	1.4 ppm	4.9 ppm
Symptomatology	0	x	xx
Mortality	0	0	+++
Growth	-	--	---
Food intake	0	-	--
Haematology	0	0	0
Urinary amorphous material	0	0	+
Urinary crystals	0	0	-
Organ weights			
Lungs	0	0	++
Heart	0	0	+
Kidneys	0	0	+
Adrenals	0	0	+++
Gross pathology			
Lungs	0	0	x
Histopathology			
Nasal cavity	x		xxx
Larynx	0	0	xx
Trachea	0	0	xxx
Bronchi + lungs	0	0	xxx

0 = Not affected, x + slightly affected, xx = moderately affected, xxx = severely affected; + = slightly increased, ++ = moderately increased, +++ = markedly increased; - slightly decreased, -- = moderately decreased, --- = markedly decreased.

Section A6.4.3(07) **Annex Point IIA6.4**		**Repeated dose toxicity** Continuous Sub-chronic Inhalation in Rats	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Lyon J.P., Jenkins L.J., Jones R.A., Coon R.A., Siegel J. (1970), Repeated and Continuous Exposure of Laboratory Animals to Acrolein, Toxicology and Applied Pharmacolgy, 17, 726-732 (1970).	
1.2	**Data protection**	No	
1.2.1	Data owner	Not applicable	
1.2.2	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No No guidelines were available at the time the study was performed.	
2.2	**GLP**	No GLP was not compulsory at the time the study was performed.	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Acrolein	
3.1.1	Lot/Batch number	Not specified	
3.1.2	Specification	Acrolein was obtained from K&K Laboratories Inc., Plainview, New York.	
3.1.2.1	Description	Not specified	
3.1.2.2	Purity	Acrolein was obtained with the highest purity commercially available and redistilled immediately prior to use.	
3.1.2.3	Stability	Not specified	
3.2	**Test Animals**		
3.2.1	Species	Rat, guinea pig, dog and monkey	
3.2.2	Strain	Rat: NMRI:O(SD) Sprague-Dawley Guinea pig: Princeton or Hartley [NMRI:(ASH) or FTD:Hartley] Dog: Purebred beagle Monkey: Squirrel (*Saimiri sciurea*)	
3.2.3	Source	Not specified	
3.2.4	Sex	Rat: Male and female Guinea pig: Male and female Dog: Male Monkey: Male	
3.2.5	Age/weight at study initiation	**Age** Not specified	

Section A6.4.3(07) **Annex Point IIA6.4**		**Repeated dose toxicity** Continuous Sub-chronic Inhalation in Rats		
		Mean weight: Rat: Male: 223 – 464 g Female: 284 – 312 g Guinea pig: Male: 381 – 655 g Female: 403 – 554 g Dog: Male: 8.3 – 12.9 kg Monkey: Male: 506 – 766 g		
3.2.6	Number of animals per group	Rat: 15 animals/group Guinea pig: 15 animals/group Dog: 2 animals/group Monkey: 9 animals/group		
3.2.7	Control animals	Yes		
3.3	**Administration/ Exposure**	Inhalation		
3.3.1	Duration of treatment	90 days		
3.3.2	Frequency of exposure	24 h/day for 90 days		
3.3.3	Postexposure period	Not specified		
3.3.4	**Inhalation**			
3.3.4.1	Concentrations	Nominal concentration	0.21, 0.23, 1.0, 1.8 ppm (0.48, 0.53, 2.29, 4.13 mg/m^3)	X
		Analytical concentration	The acrolein concentration was monitored several times daily but the results are not reported.	
3.3.4.2	Particle size	Not applicable		
3.3.4.3	Type or preparation of particles	Not applicable		
3.3.4.4	Type of exposure	Whole body		
3.3.4.5	Vehicle	Acrolein was diluted with a 75 % water – 25 % ethanol mixture		
3.3.4.6	Concentration in vehicle	Not specified		
3.3.4.7	Duration of exposure	24 h/day		
3.3.4.8	Controls	Not specified		
3.4	**Examinations**			
3.4.1	Observations			

Section A6.4.3(07) Annex Point IIA6.4		Repeated dose toxicity Continuous Sub-chronic Inhalation in Rats	
3.4.1.1	Clinical signs	Yes Animals were observed daily for visible toxic signs.	
3.4.1.2	Mortality	Yes Animals were observed daily for mortality.	
3.4.2	Body weight	Yes Animals were weighed before and after the experiment.	
3.4.3	Food consumption	Not specified	
3.4.4	Water consumption	Not specified	
3.4.5	Ophthalmoscopic examination	Not specified	
3.4.6	Haematology	Yes Number of animals: all animals Time points: pre and post exposure Parameters: haemoglobin concentration, packed erythrocyte volume, total leukocyte counts	
3.4.7	Clinical Chemisty	Yes Number of animals: all animals Time points: end of study Parameters: blood urea nitrogen concentration and alanine aminotransferase and aspartate aminotransferase activities, alkaline phosphatase, tyrosine aminotransferase activity	
3.4.8	Urinalysis	Not specified	
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	Not specified	
3.5.2	Gross and histopathology	Yes Number of animals: all dogs and monkeys and one half of the rats and guinea pigs Organs: Sections of heart, lung, liver, spleen and kidney were retained from all of the species, as well as trachea, brain and spinal cord from monkeys and trachea, brain, adrenal, thyroid and spinal cord from dogs.	
3.5.3	Other examinations	None specified	
3.5.4	Statistics	n/a	
3.6	**Further remarks**	For, the purpose of this paper, the 2 runs conducted at 0.21 and 0.23 ppm were combined and are reported as one experiment at 0.22 ppm.	
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	In the 1 ppm exposure the dogs and monkeys were visibly affected from the start. Ocular and nasal discharge was observed throughout the 90 days of exposure in the dogs but appeared to diminish in severity as the exposure continued. The monkeys kept their eyes closed for extended periods. The rats and guinea pigs appeared normal and unaffected throughout the experiment. At 1.8 ppm, the dogs and monkeys appeared to experience severe	

Section A6.4.3(07) Annex Point IIA6.4		Repeated dose toxicity Continuous Sub-chronic Inhalation in Rats	
		irritation as evidenced by excessive salivation and ocular discharge.	
4.1.2	Mortality	At the 0.22 ppm level, one monkey developed an infection over one eye during the 5th week and died the following week. At the 1.0 ppm level, one monkey died on day 28 exposure; the death was probably due to infection of a bite on the shoulder. There were no deaths during the 1.8 ppm exposure.	
4.2	**Body weight gain**	Both male and female rats gained weight normally at the 0.22 ppm level. The rate of gain for the rats in the 1.0 and 1.8 ppm exposure was significantly lower than controls with equivalent starting weights, using the student t-test. The weight changes in the guinea pigs, dogs and monkeys were not significant in any of the continuous exposures.	
4.3	**Food consumption and compound intake**	Not specified	
4.4	**Ophtalmoscopic examination**	Not specified	
4.5	**Blood analysis**		
4.5.1	Haematology	No significant differences were noted between the pre- and post-exposure values for leukocytes, haemoglobin, haematocrit and differential values at any of the 3 levels of continuous exposure.	
4.5.2	Clinical chemistry	No effects were specified	
4.5.3	Urinalysis	Not specified	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	Not specified	
4.6.2	Gross and histopathology	Sections of lung from 2 of the 4 dogs exposed to 0.22 ppm showed moderate emphysema, acute congestion, focal vacuolisation of the bronchiolar epithelial cells with increased secreory activity and, occasionally some degree of constriction of the bronchioles. In addition, focal subcapsular haemorrhage was present in sections of spleen from these 2 dogs but not from other animals. The other 2 dogs showed hyperplasia of the thyroid gland. Non-specific inflammatory changes were present in sections of liver, lung, kidney and heart from monkeys, guinea pigs and dogs. In the 1.0 ppm study, guinea pigs showed various degrees of pulmonary inflammation and occasional foci of liver necrosis. Three of 9 rats revealed focal liver necrosis and occasional pulmonary haemorrhage. Lesions of the liver in guinea pigs and rats appeared as rather minute foci without any specific pattern. Parasitic infestation was noted in some monkeys with involvement of the lung, kidney and liver. Bronchitis and early bronchopneumonia were noted in 1 dog. In the 1.8 ppm exposure, non-specific inflammatory changes were observed in sections of brain, heart, lung, liver and kidney from all animals. All monkeys showed squamous metaplasia and 6/9 monkeys showed basal cell hyperplasia of the trachea.the lungs from both dogs showed confluent bronchopneumonia. The morphological changes seen in tracheas of monkeys and lungs of dogs are considered to be related to the exposure.	

Section A6.4.3(07) **Annex Point IIA6.4**	**Repeated dose toxicity** Continuous Sub-chronic Inhalation in Rats	
4.7 Other	None	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	Experimental animals were exposed to acrolein vapours 24 h/day for 90 days at concentrations of 0.21, 0.23, 1.0 and 1.8 ppm (0.48, 0.53, 2.29, 4.13 mg/m^3). Each experimental group of animals contained rats, guinea pigs, monkeys and dogs. The animals were observed for toxic signs, mortality and weight changes and haematologic, biochemical, pathologic and histopathologic examinations were made on the surviving animals.	X
5.2 Results and discussion	In both of the lower level exposures the animals appeared normal throughout the studies and gained weight. In the higher level exposures the dogs and monkeys were visibly affected and abnormal weight patterns were observed in some animals. Based on outward signs, such as eye and respiratory irritation the dogs and monkeys appeared to be the most susceptible of the species exposed. Histopathological examination of the 0.22 ppm group showed rats and guinea pigs rarely demonstrating specific inflammatory changes whereas the dogs and monkeys showed effects. At the next higher level the dogs again showed focal inflammatory reactions of the lung, liver and kidneys. It was at this level that the guinea pigs showed pulmonary inflammation and both rats and guinea pigs showed focal liver necrosis.	X
5.3 Conclusion		
5.3.1 LO(A)EL	Not specified	X
5.3.2 NO(A)EL	0.23 ppm (0.53 mg/m^3)	X
5.3.3 Other	None	
5.3.4 Reliability	2	X
5.3.5 Deficiencies	Yes The study was not performed to GLP or a guideline.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	22/4/08	
Materials and Methods	3.3.4.1 & 5.1 It is stated that the results from the lower two dose groups (0.21 and 0.23 ppm) were combined for the reporting of the results into a single 0.22 ppm group.	

Section A6.4.3(07) **Annex Point IIA6.4**	**Repeated dose toxicity** Continuous Sub-chronic Inhalation in Rats	
Results and discussion	5.2 Rat 0.22 ppm: No treatment related effects 1.0 ppm: Pulmonary haemorrhage. Focal liver necrosis. Decrease in BW gain* 1.8 ppm: Non specific inflammation. Decrease in BW gain* Guinea pig 0.22 ppm: Inflammation in liver, lung, kidney, heart 1.0 ppm: Focal liver necrosis. Pulmonary inflammation 1.8 ppm: Non specific inflammation Monkey 0.22 ppm: Inflammation in liver, lung, kidney, heart 1.0 ppm: Clinical signs – closed eyes. 1.8 ppm: Non specific inflammation. Salivation and ocular discharge. Metaplasia of trachea. Dog 0.22 ppm: Inflammation in liver, lung, kidney, heart. Emphysema, congestion, bronchiolar constriction, thyroid hyperplasia. 1.0 ppm: Clinical signs – ocular and nasal discharge. Inflammation. Bronchiolar effects. 1.8 ppm: Non specific inflammation. Non specific inflammation. Salivation and ocular discharge. Broncho pneumonia.	
Conclusion	Overall, a NOAEL was only determined in rats but not in dogs, guinea pigs and monkeys. Consequently, the overall conclusion from the study is that a NOAEL could not be identified LO(A)EL: 0.22 ppm NO(A)EL: < 0.22 ppm	
Reliability	3	
Acceptability	Acceptable. As this study is one of a number submitted to satisfy this endpoint, there is considered to be sufficient weight of evidence to regard it as being acceptable.	
Remarks		
	COMMENTS FROM ... *(specify)*	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Table A6_3-1. Results *(specify)* of repeated dose toxicity study

Parameter	**Control**		**low dose (0.22 ppm, 0.5 mg/m3)**		**medium dose (1.0 ppm, 2.29 mg/m3)**		**high dose (1.8 ppm, 4.18 mg/m3)**		**dose-response** +/-	
	m[a]	**f[a]**	**m[a]**	**f[a]**	**m[a]**	**f[a]**	**m[a]**	**f[a]**	**m**	**f**
number of animals examined										
rat			15	15	7	8	7	8		
guinea pig			15	15	7	8	6	9		
dog			4		2		2			
monkey			17		8		9			
Mortality:										
monkey			1 (at week 5)		1 (day 28)					
clinical signs*:										
ocular, nasal discharge:										
dog					↑					
monkey					↑					
excess salivation, ocular discharge:										
dog							↑			
monkey							↑			
body weight:										
rat			↑	↑	↑	↑	↓	↓		
guinea pig			↑	↑	↑	↑	↑	↑		
dog			↑		↑		↑			
monkey			↓		↓		↓			

** specify effects; for different organs give special findings in the order organ weight, gross pathology and microscopic pathology if there are effects*

[a] *give number of animals affected/total number of animals, percentage, or just ↑ or ↓ for increased or decreased*